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REGULAR RESEARCH ARTICLE

Resolvin D1 and D2 Reverse Lipopolysaccharide-Induced Depression-Like Behaviors Through the mTORC1 Signaling Pathway

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Abstract

Background: Resolvin D1 and D2 are bioactive lipid mediators that are generated from docosahexaenoic acid. Although recent preclinical studies suggest that these compounds have antidepressant effects, their mechanisms of action remain unclear.

Methods: We investigated mechanisms underlying the antidepressant effects of resolvin D1 and resolvin D2 in lipopolysaccharide (0.8 mg/kg, i.p.)-induced depression model mice using a tail suspension test.

Results: I.c.v. infusion of resolvin D1 (10 ng) and resolvin D2 (10 ng) produced antidepressant effects; these effects were significantly blocked by a resolvin D1 receptor antagonist WRW4 (10 µg, i.c.v.) and a resolvin D2 receptor antagonist O-1918 (10 µg, i.c.v.), respectively. The mammalian target of rapamycin complex 1 inhibitor rapamycin (10 mg/kg, i.p.) and a mitogen-activated protein kinase kinase inhibitor U0126 (5 µg, i.c.v.) significantly blocked the antidepressant effects of resolvin D1 and resolvin D2. An AMPA receptor antagonist NBQX (10 mg/kg, i.p.) and a phosphoinositide 3-kinase inhibitor LY294002 (3 µg, i.c.v.) blocked the antidepressant effects of resolvin D1 significantly, but not of resolvin D2. Bilateral infusions of resolvin D1 (0.3 ng/side) or resolvin D2 (0.3 ng/side) into the medial prefrontal cortex or dentate gyrus of the hippocampus produced antidepressant effects.

Conclusions: These findings demonstrate that resolvin D1 and resolvin D2 produce antidepressant effects via the mammalian target of rapamycin complex 1 signaling pathway, and that the medial prefrontal cortex and dentate gyrus are important brain regions for these antidepressant effects. These compounds and their receptors may be promising targets for the development of novel rapid-acting antidepressants, like ketamine and scopolamine.

Keywords: dentate gyrus, depression, resolvin, medial prefrontal cortex, mTORC1

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Significance Statement

There is a continuing unmet need for more effective and rapid-acting antidepressants, because currently available antidepressants have significant limitations, including delayed onset and low efficacy. Here, we demonstrate that docosahexaenoic acid-derived endogenous lipid mediators, resolvin D1 (RvD1) and D2 (RvD2), produce antidepressant effects through mechanisms (activation of mTORC1 signaling) similar to those underlying the rapid antidepressant effects of the NMDA receptor antagonist ketamine and the nonselective muscarinic receptor antagonist scopolamine. We also show that the medial prefrontal cortex and dentate gyrus of the hippocampus are important brain regions for the antidepressant effects of RvD1 and RvD2. Our findings suggest that RvD1 and RvD2 may be promising targets for the development of novel, rapid-acting antidepressants.

Introduction

Major depressive disorder is one of the most common psychiatric illnesses, leading to enormous personal and socioeconomic burdens (Greenberg et al., 2015). Because currently available monoamine-based antidepressants have significant limitations, including delayed onset of treatment response (weeks to months) and low efficacy (approximately one-third of depressed patients fail to respond to 2 or more antidepressants and are characterized as having treatment-resistant depression) (Trivedi et al., 2006), there is a significant unmet medical need for more effective and rapid-acting antidepressants. Recent clinical studies have shown that ketamine (an NMDA receptor antagonist) and scopolamine (a nonselective muscarinic receptor antagonist) produce rapid antidepressant effects (within hours) even in treatment-resistant depressed patients (Zarate et al., 2006; Ellis et al., 2014). Preclinical studies have revealed that the rapid antidepressant effects of these drugs are mediated by activation of mammalian target of rapamycin complex 1 (mTORC1), a downstream target of phosphoinositide 3-kinase (PI3K)/Akt and mitogen-activated protein kinase kinase (MEK)/extracellular signal-regulated kinase (ERK) signaling, in brain regions such as the medial prefrontal cortex (mPFC) and hippocampus (Li et al., 2010; Voleti et al., 2013; Duman et al., 2016). These findings suggest that compounds capable of activating mTORC1 signaling may have potential to function as rapid-acting antidepressants.

Resolvins are bioactive lipid mediators that are generated from the n-3 polyunsaturated fatty acids docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). Resolvin D1 (RvD1) and resolvin D2 (RvD2) are DHA-derived D-series resolvins. RvD1 activates formyl peptide receptor 2/lipoxin A₄ receptor (FPR2/ALX) and human GPR32 (the murine counterpart of human GPR32 remains unknown) (Haitina et al., 2009; Krishnamoorthy et al., 2010). Stimulation of FPR2/ALX by RvD1 or lipoxin A₄ activates the PI3K/Akt and MEK/ERK pathways (Odusanwo et al., 2012; Hodges et al., 2017). Recently, GPR18 has been identified as a RvD2 receptor (Chiang et al., 2015), and stimulation of GPR18 also activates the PI3K/Akt and MEK/ERK signaling pathways (Penumarti and Abdel-Rahman, 2014). These findings suggest the possibility that RvD1 and RvD2 may produce antidepressant effects via activation of PI3K/Akt and/or MEK/ERK signaling pathways, and then activation of their downstream target, mTORC1, similar to the rapid-acting antidepressants ketamine and scopolamine. Thus, the aim of the present study was to test this possibility. First, we examined whether i.c.v. infusions of RvD1 and RvD2 produced antidepressant effects through FPR2/ALX and GPR18, respectively, using lipopolysaccharide (LPS)-induced depression model mice (O'Connor et al., 2009; Kang et al., 2011). Second, we examined the involvement of mTORC1 and upstream signaling molecules, AMPA receptors,

PI3K/Akt, and MEK/ERK, in the antidepressant effects of RvD1 and RvD2. Finally, we examined the roles of the mPFC and the dentate gyrus (DG) of the hippocampus in their antidepressant effects.

Materials and Methods

Animals

Male BALB/c mice (8–12 weeks old, Japan SLC) were group-housed (3–5/cage). The mice were maintained at a constant ambient temperature (23±1°C) under a 12-h-light/-dark cycle (light on 7:00 AM) with food and water available *ad libitum*. All experiments were performed with the approval of the Institutional Animal Care and Use Committees at Hokkaido University and Kanazawa University.

Drugs

RvD1 and RvD2 were obtained as a solution in 100% ethanol from Cayman Chemical and stored at -80°C. These solutions were diluted with sterile phosphate-buffered saline (PBS) to achieve a final ethanol concentration of 2% immediately before use while minimizing exposure to light. LPS (serotype 0127:B8; Sigma) was dissolved freshly in sterile saline. WRW4 (an FPR2/ALX antagonist; Tocris) was dissolved in sterile PBS. O-1918 (a GPR18 antagonist; Abcam) was dissolved initially in dimethyl sulfoxide (DMSO) and then diluted with sterile PBS containing Tween 80 and ethanol (final concentrations of DMSO, Tween 80, and ethanol were 10%, 5%, and 2%, respectively). LY294002 (a PI3K inhibitor; Cayman Chemical) was dissolved initially in DMSO and then diluted with sterile PBS containing ethanol (final concentrations of DMSO and ethanol were 10% and 2%, respectively). U0126 (a MEK inhibitor; LC Laboratories) was dissolved initially in DMSO and then diluted with sterile PBS containing Tween 80 and ethanol (final concentrations of DMSO, Tween 80, and ethanol were 5%, 5%, and 2%, respectively). Rapamycin (an mTORC1 inhibitor; LC Laboratories) was dissolved initially in DMSO and then diluted with sterile saline containing Tween 80 (final concentrations of DMSO and Tween 80 were both 5%). NBQX disodium salt (an AMPA receptor antagonist; Enzo Life Sciences) was dissolved in sterile saline.

LPS Challenge

LPS (0.8 mg/kg) or saline was injected i.p. into each mouse at a volume of 10 mL/kg. The dose of LPS was selected because it could induce depression-like behaviors 24 h after LPS challenge without reducing locomotor activity (O'Connor et al., 2009; Kang

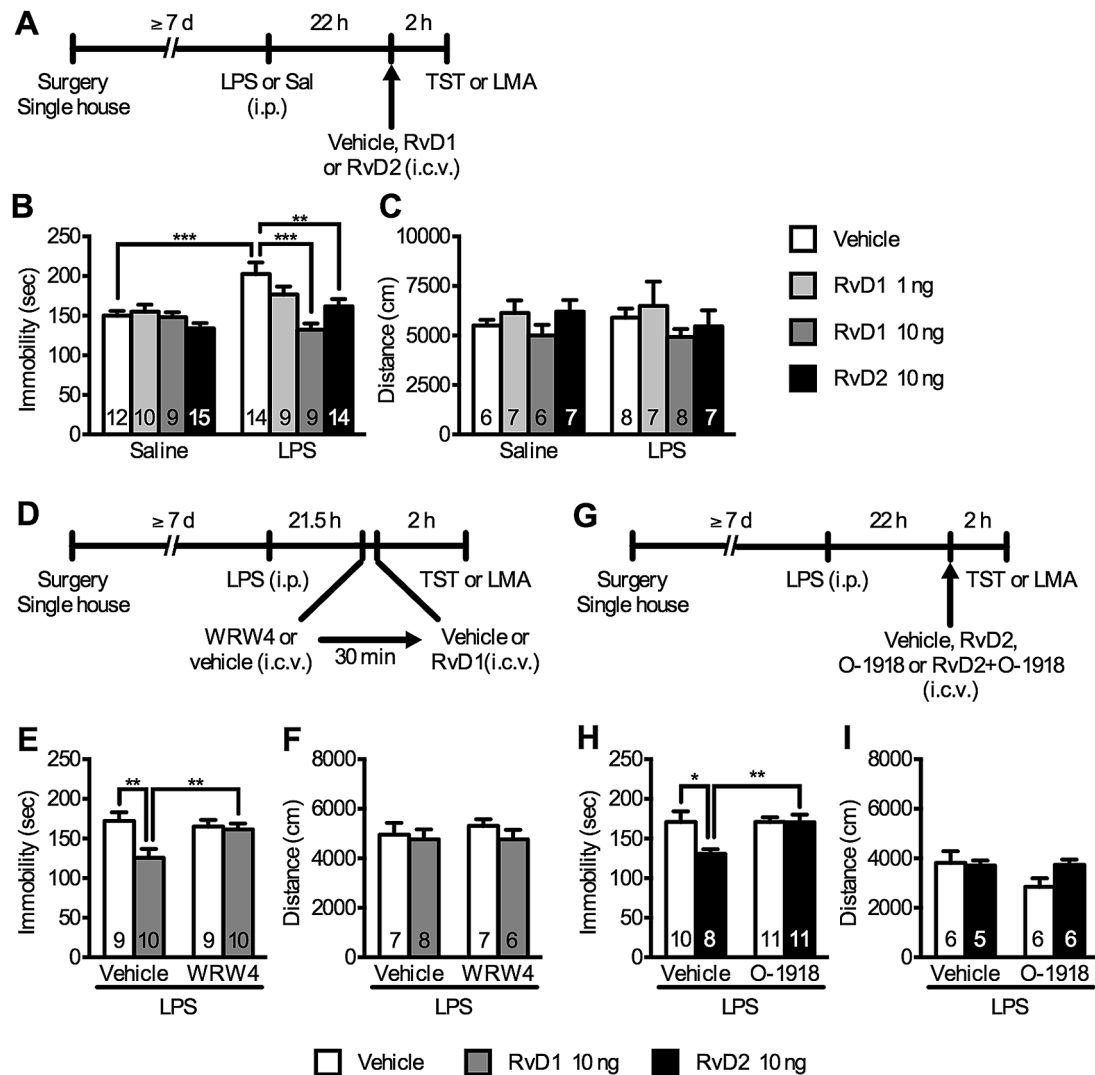


Figure 1. Resolvin D1 (RvD1) and resolvin D2 (RvD2) produced antidepressant effects in the lipopolysaccharide (LPS)-induced depression model through formyl peptide receptor 2/lipoxin A₂ receptor (FPR2/ALX) and GPR18, respectively. (A, D, G) Experimental timeline for LPS challenge (0.8 mg/kg, i.p.), i.c.v. injection, and behavioral testing. (B) Immobility time in the tail suspension test (TST) (interaction: $F_{3,84} = 3.879$, $P = .0119$, $n = 9-15$). (C) Locomotor activity (LMA) (interaction: $F_{3,48} = 0.3034$, $P = .8228$, LPS: $F_{1,48} = 0.001887$, $P = .9655$, treatment: $F_{3,48} = 1.324$, $P = .2774$, $n = 6-8$). (E) Immobility time in the TST (interaction: $F_{1,34} = 5.203$, $P = .0289$, $n = 9-10$). (F) LMA (interaction: $F_{1,24} = 0.1980$, $P = .6603$, WRW4: $F_{1,24} = 0.2042$, $P = .6554$, treatment: $F_{1,24} = 0.8458$, $P = .3669$, $n = 6-8$). (H) Immobility time in the TST (interaction: $F_{1,19} = 2.150$, $P = .1589$, O-1918: $F_{1,19} = 1.973$, $P = .1763$, treatment: $F_{1,19} = 1.365$, $P = .2571$, $n = 5-6$). Data are expressed as means \pm SEM. * $P < .05$, ** $P < .01$, *** $P < .001$ (2-way ANOVA followed by the Newman-Keuls posthoc test).

et al., 2011). In the present study, behavioral tests were performed 24 hours after LPS challenge.

Surgery and Drug Treatments

Mice were anesthetized with chloral hydrate (400 mg/kg, i.p.). The mice were implanted with a 25-gauge stainless-steel guide cannula (o.d., 0.5 mm; i.d., 0.22 mm) above the infusion sites in either the lateral ventricle (-0.3 mm rostral, 1.0 mm lateral, 2.0 mm ventral to bregma; unilateral), mPFC (1.9 mm rostral, ± 0.8 mm lateral, 0.95 mm ventral to bregma, at a 20° angle, bilateral), or DG (-1.7 mm rostral, ± 1.1 mm lateral, 0.5 mm ventral to bregma, bilateral) (Franklin and Paxinos, 2007). After surgery, mice were housed individually and allowed to recover for at least 7 days. The i.c.v., intra-mPFC, and intra-DG infusions of drugs were carried out 22 hours after LPS challenge.

These local infusions of drugs were done in conscious mice gently held by the experimenter's hand. For i.c.v. infusions, a 33-gauge stainless-steel injection cannula (o.d., 0.2 mm; i.d., 0.08 mm) was inserted into the guide cannula; the injection cannula protruded 0.8 mm from the tip of the guide cannula to reach the lateral ventricle. The injection cannula was attached to a Hamilton microsyringe mounted on a microinfusion pump (Eicom) via PE8 tubing. RvD1 (1 or 10 ng), RvD2 (10 ng), O-1918 (10 μ g), LY294002 (3 μ g), U0126 (5 μ g), or vehicle was administered in a 5- μ L volume at a rate of 2.5 μ L/min. WRW4 (10 μ g, i.c.v.) was infused in a 2- μ L volume at a rate of 2 μ L/min 30 minutes prior to i.c.v. infusion of RvD1. After i.c.v. infusions, the injection cannula was kept in place for another 1 minute to allow for diffusion. For intra-mPFC and intra-DG infusions, 33-gauge stainless-steel injection cannulae were inserted bilaterally into the guide cannulae; the injection cannulae protruded 1.0 and

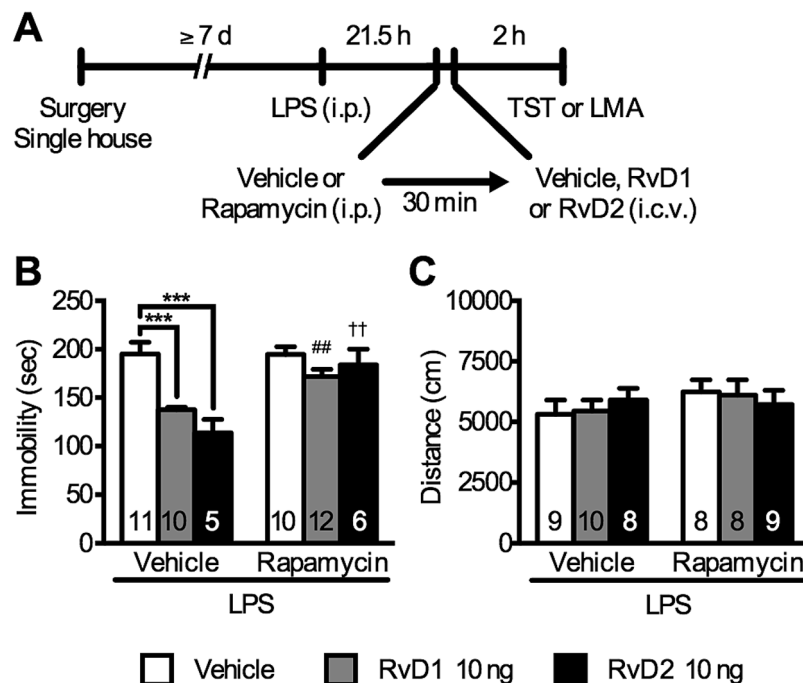


Figure 2. Resolvin D1 (RvD1) and resolvin D2 (RvD2) produced antidepressant effects through the mammalian target of rapamycin complex 1 (mTORC1) signaling pathway. (A) Experimental timeline for testing the role of mTORC1 in the antidepressant effects of RvD1 and RvD2. Rapamycin (an mTORC1 inhibitor, 10 mg/kg, i.p.) was injected 30 minutes prior to i.c.v. infusion of RvD1 or RvD2. (B) Immobility time in the tail suspension test (TST) (interaction: $F_{2,46} = 5.273$, $P = .0085$, $n = 5-12$). (C) Locomotor activity (LMA) (interaction: $F_{2,46} = 0.5457$, $P = .5831$, rapamycin: $F_{1,46} = 1.102$, $P = .2993$, treatment: $F_{2,46} = 0.003064$, $P = .9969$, $n = 8-10$). Data are expressed as means \pm SEM. *** $P < .001$ relative to vehicle; ** $P < .01$ relative to vehicle + RvD1; † $P < .01$ relative to vehicle + RvD2 (2-way ANOVA followed by the Newman-Keuls posthoc test).

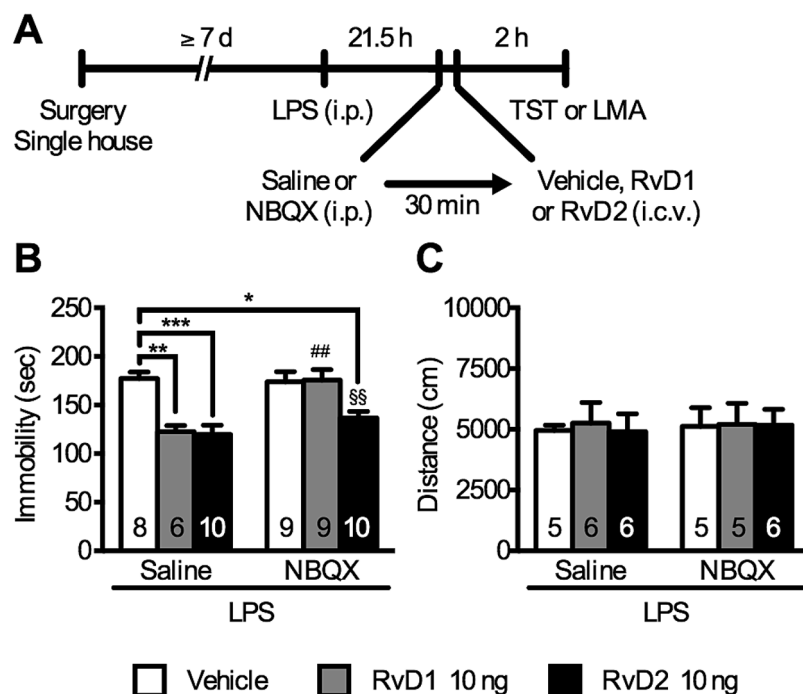


Figure 3. AMPA receptor-mediated glutamatergic transmission is required for the antidepressant effect of resolvin D1 (RvD1), but not of resolvin D2 (RvD2). (A) Experimental timeline for testing the role of glutamatergic transmission in the antidepressant effects of RvD1 and RvD2. NBQX (an AMPA receptor antagonist, 10 mg/kg, i.p.) was injected 30 minutes prior to i.c.v. infusion of RvD1 or RvD2. (B) Immobility time in the tail suspension test (TST) (interaction: $F_{2,46} = 4.459$, $P = .0170$, $n = 6-10$). (C) Locomotor activity (LMA) (interaction: $F_{2,27} = 0.02636$, $P = .9740$, NBQX: $F_{1,27} = 0.04599$, $P = .8318$, treatment: $F_{2,27} = 0.04579$, $P = .9553$, $n = 5$ or 6). Data are expressed as means \pm SEM. * $P < .05$, ** $P < .01$, *** $P < .001$ relative to vehicle; † $P < .01$ relative to saline + RvD1; ‡ $P < .01$ relative to NBQX + vehicle (2-way ANOVA followed by the Newman-Keuls posthoc test).

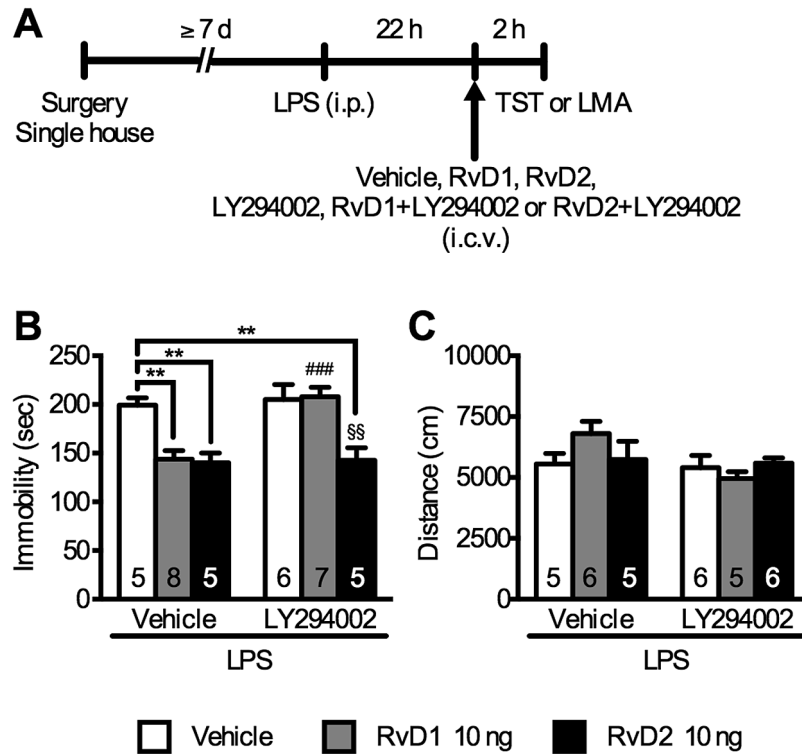


Figure 4. Phosphoinositide 3-kinase (PI3K)/Akt signaling pathway is required for the antidepressant effect of resolvin D1 (RvD1), but not of resolvin D2 (RvD2). (A) Experimental timeline for testing the role of PI3K/Akt signaling pathway in the antidepressant effects of RvD1 and RvD2. LY294002 (a PI3K inhibitor, 3 μ g, i.c.v.) was infused simultaneously with RvD1 or RvD2. (B) Immobility time in the tail suspension test (TST) (interaction: $F_{2,30} = 5.340$, $P = .0104$, $n = 5-8$). (C) Locomotor activity (LMA) (interaction: $F_{2,27} = 2.161$, $P = .1347$, LY294002: $F_{1,27} = 3.422$, $P = .0753$, treatment: $F_{2,27} = 0.3544$, $P = .7048$, $n = 5$ or 6). Data are expressed as means \pm SEM. ** $P < .01$ relative to vehicle; *** $P < .001$ relative to vehicle + RvD1; §§ $P < .01$ relative to LY294002 + vehicle (2-way ANOVA followed by the Newman-Keuls posthoc test).

1.5 mm, respectively, from the tip of guide cannulae to reach the mPFC (1.9 mm rostral, ± 0.25 mm lateral, 2.0 mm ventral to bregma) and DG (-1.7 mm rostral, ± 1.1 mm lateral, 2.0 mm ventral to bregma). RvD1 (0.3 ng/side), RvD2 (0.3 ng/side), or vehicle was injected bilaterally in a 0.15- μ L volume at a rate of 0.15 μ L/min, and the injection cannulae were kept in place for another 1 minute. NBQX (10 mg/kg) and rapamycin (10 mg/kg) were administered i.p. 30 min prior to i.c.v. infusion. The doses of the antagonists and enzyme inhibitors used in the present study were determined based on pilot experiments and previous reports demonstrating significant inhibition of the effect of each target molecule (Maeng et al., 2008; Zeni et al., 2012; Osborn et al., 2013).

Tail Suspension Test

Each mouse was suspended with its tail fixed to a hook by a small piece of adhesive tape. Using an activity measuring and recording system equipped with an infrared detector (Supermex, CompACT FSS, Muromachi Kikai), the duration of immobility was measured automatically for 6 minutes. Mice that climbed their tail during the test period were excluded from the analyses.

Locomotor Activity Test

Different cohorts of mice were used for the locomotor activity test. Each mouse was placed in a gray polyvinyl chloride chamber (40 \times 40 \times 35 cm) for 10 minutes. The total distance (cm) traveled during this period was automatically measured using the EthoVision video tracking system (Noldus Information Technology).

Histology

After behavioral tests, histological analyses were performed. Mice were euthanized by cervical dislocation, and the brains were removed rapidly and frozen on powdered dry ice. Coronal sections (50 μ m) were prepared on a cryostat, thaw-mounted on slides, and stained with thionin to confirm the sites of infusion. Mice with incorrect infusion placements were excluded from the analyses.

Statistical Analyses

Data are expressed as means \pm SEM. Data were analyzed by 2-way ANOVA followed by the Newman-Keuls posthoc test using the GraphPad Prism 6 software (GraphPad Software). Differences with $P < .05$ were considered statistically significant.

Results

Antidepressant Effects of RvD1 and RvD2 via FPR2/ALX and GPR18, Respectively

To examine the antidepressant effects of RvD1 and RvD2, we used the LPS-induced depression model mice. Mice were i.p. injected with LPS (0.8 mg/kg) or saline, and then 22 hours later, i.c.v. infusion of RvD1 (1 or 10 ng), RvD2 (10 ng), or vehicle (2% ethanol/PBS) was carried out (Figure 1A). The tail suspension test or locomotor activity test was conducted 2 hours after i.c.v. infusion. In the tail suspension test, LPS challenge significantly increased immobility in vehicle-infused mice, and this depression-like behavior was significantly alleviated by i.c.v. infusion of RvD1 (10 ng) or RvD2

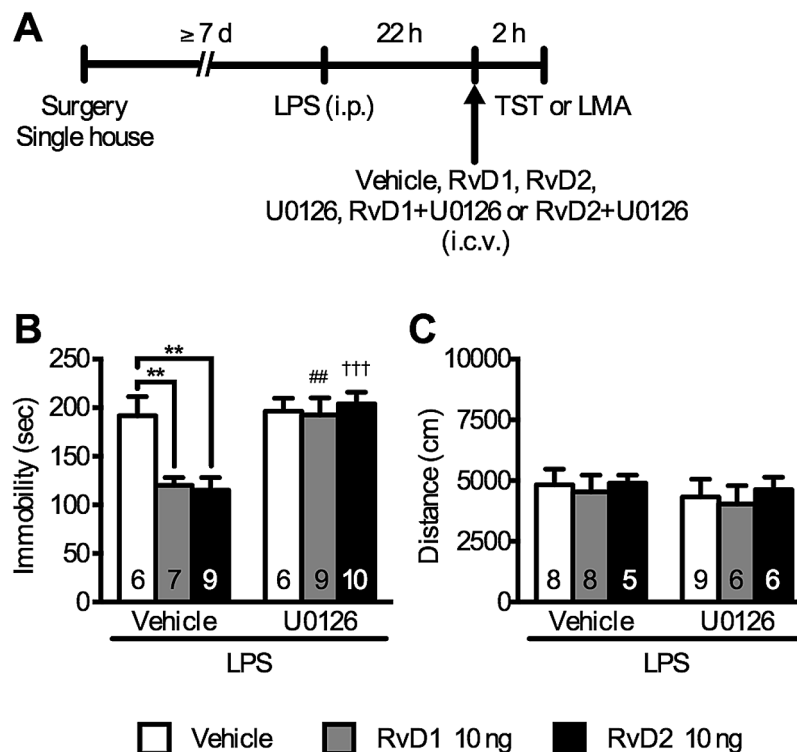


Figure 5. Mitogen-activated protein kinase kinase (MEK)/extracellular signal-regulated kinase (ERK) signaling pathway is required for the antidepressant effects of resolvin D1 (RvD1) and resolvin D2 (RvD2). (A) Experimental timeline for testing the role of MEK/ERK signaling pathway in the antidepressant effects of RvD1 and RvD2. U0126 (a MEK inhibitor, 5 μ g, i.c.v.) was infused simultaneously with RvD1 or RvD2. (B) Immobility time in the tail suspension test (TST) (interaction: $F_{2,41} = 4.241$, $P = .0212$, $n = 6-10$). (C) Locomotor activity (LMA) (interaction: $F_{2,36} = 0.01893$, $P = .9813$, U0126: $F_{1,36} = 0.5755$, $P = .4530$, treatment: $F_{2,36} = 0.2122$, $P = .8098$, $n = 5-9$). Data are expressed as means \pm SEM. ** $P < .01$ relative to vehicle; ## $P < .01$ relative to vehicle + RvD1; +++ $P < .001$ relative to vehicle + RvD2 (2-way ANOVA followed by the Newman-Keuls posthoc test).

(10 ng) (Figure 1B). RvD1 and RvD2 had no effect on immobility in saline-injected control mice. There was no effect of LPS challenge or i.c.v. infusion of RvD1 or RvD2 on locomotor activity (Figure 1C), indicating that the differences observed in the tail suspension test were not due to a general change in locomotor activity.

To investigate the role of FPR2/ALX in the antidepressant effect of RvD1, WRW4 (10 μ g; an FPR2/ALX antagonist) or vehicle (PBS) was administered 30 minutes before i.c.v. infusion of RvD1 (10 ng) (Figure 1D). In the tail suspension test, RvD1 decreased immobility significantly in vehicle-pretreated mice, but this effect was completely blocked in WRW4-pretreated mice (Figure 1E). There was no significant effect of WRW4 and RvD1 on locomotor activity (Figure 1F). These results indicated that RvD1 exerted its antidepressant effects via FPR2/ALX.

Next, we examined the role of GPR18 in the antidepressant effect of RvD2. RvD2 (10 ng) and O-1918 (10 μ g; a GPR18 antagonist) were i.c.v. infused simultaneously (Figure 1G). RvD2 decreased immobility significantly in vehicle (10% DMSO/5% Tween 80/2% ethanol/PBS)-treated mice, but this effect was completely blocked by co-infusion with O-1918 (Figure 1H). There was no effect of O-1918 and RvD2 on locomotor activity (Figure 1I). These results indicated that RvD2 produced its antidepressant effects through GPR18.

Involvement of mTORC1 Signaling Pathway in Antidepressant Effects of RvD1 and RvD2

Rapamycin (10 mg/kg; an mTORC1 inhibitor) or vehicle (5% DMSO/5% Tween 80/saline) was administered i.p. 30 minutes before i.c.v. infusion of RvD1 (10 ng), RvD2 (10 ng), or vehicle

(2% ethanol/PBS) (Figure 2A). RvD1 and RvD2 decreased immobility significantly in vehicle-pretreated mice, but this effect was completely blocked by pretreatment with rapamycin (Figure 2B). These treatments did not affect locomotor activity (Figure 2C). Together, these findings indicate that activation of mTORC1 signaling mediated the antidepressant effects of RvD1 and RvD2.

Involvement of AMPA Receptors in Antidepressant Effect of RvD1, but Not of RvD2

NBQX (10 mg/kg; an AMPA receptor antagonist) or vehicle (saline) was administered i.p. 30 minutes before i.c.v. infusion of RvD1 (10 ng), RvD2 (10 ng), or vehicle (2% ethanol/PBS) (Figure 3A). RvD1 and RvD2 decreased immobility significantly in vehicle-pretreated mice. Pretreatment with NBQX blocked the antidepressant effect of RvD1 significantly, but not of RvD2 (Figure 3B). Locomotor activity was not affected by any of the drugs (Figure 3C). These results indicated that activation of AMPA receptors was required for the antidepressant effect of RvD1, but not of RvD2.

Involvement of PI3K/Akt Signaling Pathway in Antidepressant Effect of RvD1, but Not of RvD2

LY294002 (3 μ g; a PI3K inhibitor) was i.c.v. coadministered with RvD1 (10 ng) or RvD2 (10 ng) (Figure 4A). RvD1 and RvD2 decreased immobility significantly compared with the vehicle (10% DMSO/2% ethanol/PBS)-treated group. Coadministration of LY294002 blocked the antidepressant effect of RvD1 significantly, but not of RvD2 (Figure 4B). Locomotor activity was not

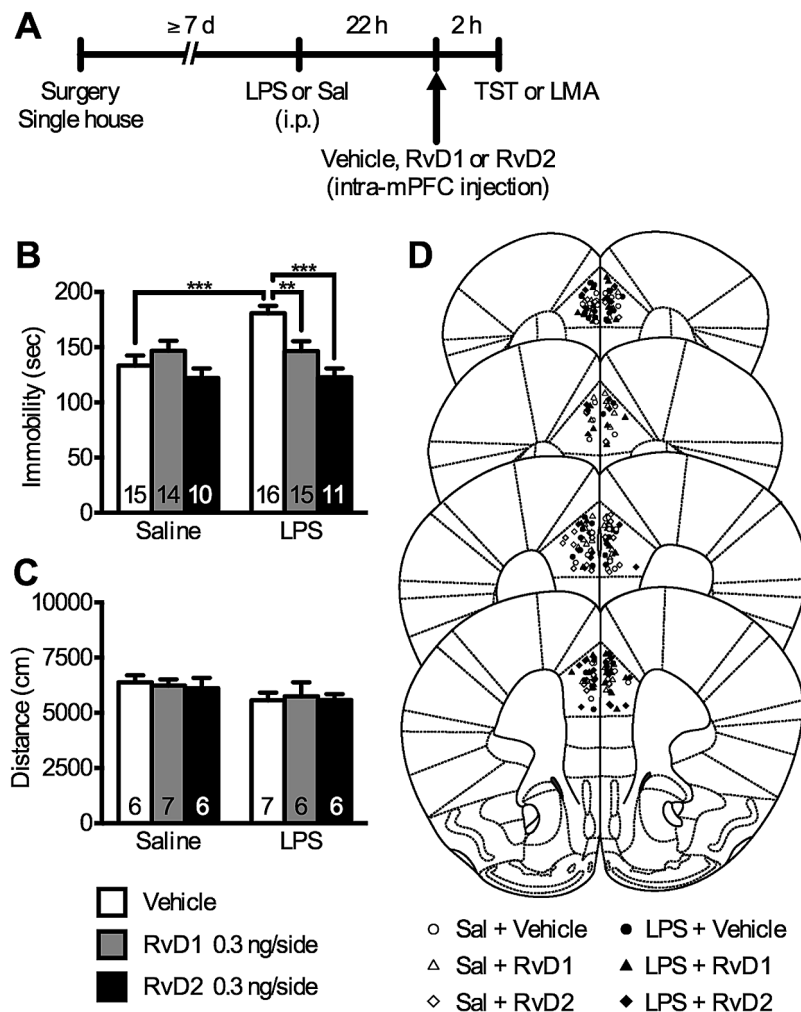


Figure 6. Intra-medial prefrontal cortex (mPFC) infusion of resolvins D1 (RvD1) and resolvins D2 (RvD2) produced antidepressant effects. (A) Experimental timeline for lipopolysaccharide (LPS) challenge (0.8 mg/kg, i.p.), intra-mPFC infusion (RvD1 0.3 ng/side, RvD2 0.3 ng/side or vehicle), and behavioral testing. (B) Immobility time in the tail suspension test (TST) (interaction: $F_{2,75} = 5.448$, $P = .0062$, $n = 10-16$). (C) Locomotor activity (LMA) (interaction: $F_{2,32} = 0.1079$, $P = .8981$, LPS: $F_{1,32} = 3.629$, $P = .0658$, treatment: $F_{2,32} = 0.07019$, $P = .9324$, $n = 6$ or 7). (D) Schematic representation of mPFC infusion sites (plates are from Franklin and Paxinos, 2007). Data are expressed as means \pm SEM. ** $P < .01$, *** $P < .001$ (2-way ANOVA followed by the Newman-Keuls posthoc test).

affected by any of the drugs (Figure 4C). These results indicated that the antidepressant effects of RvD1, but not of RvD2, were mediated by the PI3K/Akt signaling pathway.

Involvement of MEK/ERK Signaling Pathway in Antidepressant Effects of RvD1 and RvD2

U0126 (5 μ g; a MEK inhibitor) was i.c.v. coadministered with RvD1 (10 ng) or RvD2 (10 ng) (Figure 5A). RvD1 and RvD2 decreased immobility significantly compared with the vehicle (5% DMSO/5% Tween 80/2% ethanol/PBS)-treated group. Coadministration of U0126 blocked the antidepressant effects of both RvD1 and RvD2 significantly (Figure 5B). Locomotor activity was not affected by any of the drugs (Figure 5C). These results indicated that the antidepressant effects of both RvD1 and RvD2 were mediated by the MEK/ERK signaling pathway.

Roles of the mPFC and DG in Antidepressant Effects of RvD1 and RvD2

Intra-mPFC infusion of vehicle (2% ethanol/PBS), RvD1 (0.3 ng/side), or RvD2 (0.3 ng/side) was carried out 2 hours before

behavioral testing (Figure 6A). Intra-mPFC infusion of RvD1 and RvD2 reversed the LPS-induced increase in immobility significantly but had no effect on immobility in saline-injected mice (Figure 6B). Locomotor activity was not affected by intra-mPFC infusion of these compounds (Figure 6C). Intra-DG infusion of vehicle (2% ethanol/PBS), RvD1 (0.3 ng/side), or RvD2 (0.3 ng/side) was carried out 2 hours before behavioral testing (Figure 7A). Intra-DG infusion of RvD1 and RvD2 reversed the LPS-induced increase in immobility significantly but had no effect on immobility in saline-injected mice (Figure 7B). Locomotor activity was not affected by intra-DG infusion of these compounds (Figure 7C). These data demonstrated that the mPFC and the DG play an important role in the antidepressant effects of RvD1 and RvD2.

Discussion

The present study demonstrated that (1) i.c.v. infusions of RvD1 and RvD2 exerted antidepressant effects in the LPS-induced depression model mice, (2) the antidepressant effects of RvD1 and RvD2 were blocked by pretreatment with rapamycin, and (3) local injection of RvD1 and RvD2 into the mPFC or DG exerted

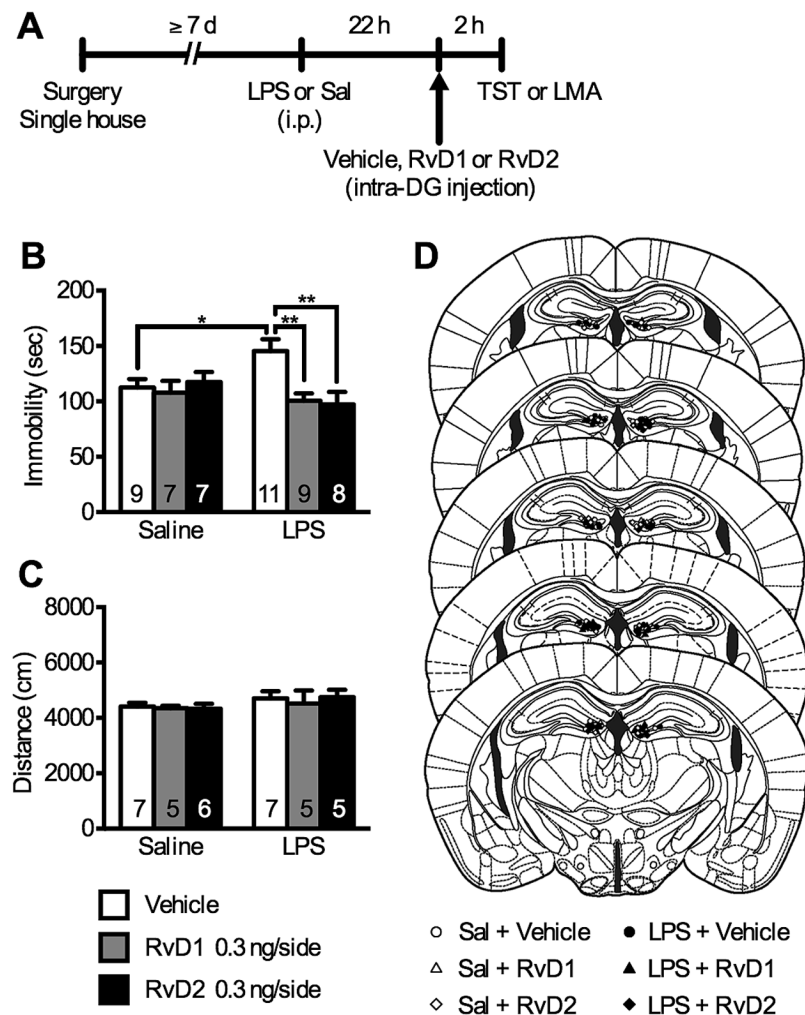


Figure 7. Intra-dentate gyrus (DG) infusion of resolvin D1 (RvD1) and resolvin D2 (RvD2) produced antidepressant effects. (A) Experimental timeline for lipopolysaccharide (LPS) challenge (0.8 mg/kg, i.p.), intra-mDG infusion (RvD1 0.3 ng/side, RvD2 0.3 ng/side or vehicle), and behavioral testing. (B) Immobility time in the tail suspension test (TST) (interaction: $F_{2,45} = 4.432$, $P = .0175$, $n = 7-11$). (C) Locomotor activity (LMA) (interaction: $F_{2,29} = 0.1067$, $P = .8992$, LPS: $F_{1,29} = 1.957$, $P = .1725$, treatment: $F_{2,29} = 0.1267$, $P = .8814$, $n = 5-7$). (D) Schematic representation of DG infusion sites (plates are from Franklin and Paxinos, 2007). Data are expressed as means \pm SEM. * $P < .05$, ** $P < .01$ (2-way ANOVA followed by the Newman-Keuls posthoc test).

antidepressant effects. Although previous studies reported the antidepressant effects of peripherally administered RvD1 and RvD2 in rodent models of myocardial infarct- (Gilbert et al., 2014) and fibromyalgia- (Klein et al., 2014) associated depression, to our knowledge, these are the first reported results to suggest that RvD1 and RvD2 act on brain regions, such as the mPFC and DG, to exert antidepressant effects via activation of mTORC1 signaling, similar to rapid-acting antidepressants, such as ketamine and scopolamine (Li et al., 2010; Voleti et al., 2013; Duman et al., 2016).

Low-dose ketamine and scopolamine preferentially block NMDA and M1 acetylcholine receptors on GABAergic interneurons, respectively, leading to increased glutamate release in the mPFC via presynaptic disinhibition and activation of AMPA receptors (Moghaddam et al., 1997; Voleti et al., 2013; Duman et al., 2016; Wohleb et al., 2016b). AMPA receptor activation results in elevation of the number and function of spine synapses in the mPFC through activity-dependent BDNF release and subsequent activation of TrkB and the downstream mTORC1 pathway to exert their antidepressant effects (Duman et al., 2016). The present study demonstrates the involvement of mTORC1, AMPA receptors, PI3K/Akt, and MEK/ERK signaling pathways in the

antidepressant effects of RvD1, suggesting that the underlying mechanism of the antidepressant effect of RvD1 may be similar to those of ketamine and scopolamine. In contrast, the antidepressant effect of RvD2 was not blocked by NBQX or LY294002, whereas the effect was fully blocked by U0126 and rapamycin. These results suggest that RvD2 and its receptor, GPR18, require activation of the MEK/ERK pathway and downstream mTORC1 signaling, but not AMPA receptor-mediated glutamatergic transmission or PI3K/Akt signaling, to exert an antidepressant effect. Further studies are needed to fully determine the signaling pathway(s) for the antidepressant effects of RvD1 and RvD2.

Although the mechanisms underlying the pathophysiology and treatments of depression remain unclear, there is growing evidence that inflammatory responses, including neutrophil infiltration into the brain and increased expression of proinflammatory mediators, are involved in the neurobiology of depression, and that antiinflammatory agents produce antidepressant effects (Aguilar-Valles et al., 2014; He et al., 2016; Wohleb et al., 2016a). RvD1 and RvD2 exert antiinflammatory and pro-resolving actions in various animal models of peripheral inflammation (Ji et al., 2011; Serhan, 2014). Because the LPS-induced depression model is an inflammation-based model

(O'Connor et al., 2009; Kang et al., 2011), RvD1 and RvD2 may alleviate the LPS-induced depression-like behaviors via anti-inflammatory actions, such as the inhibition of neutrophil chemotaxis and reduced expression of proinflammatory mediators. However, a recent study showed that repeated i.c.v. infusion of RvD2 increased not only an antiinflammatory cytokine, IL-10, but also a proinflammatory cytokine, IL-6, in the hypothalamus of high-fat diet-fed mice (Pascoal et al., 2017). They also demonstrated that repeated i.c.v. infusion of RvD2 did not reduce high-fat diet-induced elevation of IL-1 β or TNF- α mRNA levels in the hypothalamus. These findings suggest that the anti-inflammatory effects of RvD1 or RvD2 in the brain may be limited. Further studies are needed to determine whether antiinflammatory and/or pro-resolving properties of RvD1 and RvD2 contribute to their antidepressant effects.

The mPFC and DG are the brain regions implicated in the pathophysiology of depression and the actions of antidepressants (Warner-Schmidt and Duman, 2006; Duman et al., 2016). In this study, we demonstrated that intra-mPFC and intra-DG injections of RvD1 and RvD2 exerted antidepressant effects, suggesting that these brain regions are sites of action of these compounds. A recent study showed that Alox15, the enzyme that metabolizes DHA to 17S-hydroxy-DHA, a precursor molecule of RvD1 and RvD2, is expressed highly in the PFC and hippocampus, and localized at neuronal dendrites (Shalini et al., 2017). Inhibition or knockdown of Alox15 in the PFC significantly decreased RvD1 levels, blocked long-term potentiation in the hippocampo-PFC pathway, and impaired spatial working memory. These findings indicate that endogenous 17S-hydroxy-DHA and its metabolites, RvD1 and RvD2, play an important role in neuronal functions in the PFC and hippocampus. To determine the precise cellular mechanisms underlying the effects of RvD1 and RvD2 in these brain regions, further studies, including histological analyses for expression patterns of FPR2/ALX and GPR18 and electrophysiological analyses of the effects of RvD1 and RvD2 on synaptic transmission, are needed. Nevertheless, the current study provides evidence that RvD1 and RvD2 ameliorate depression-like behaviors through the mTORC1 signaling pathway, suggesting that these compounds and their receptors may be promising targets for the development of novel, rapid-acting antidepressants.

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Statement of Interest

None.

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